JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 250–253 Vol. 41, No. 1
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.1.250–253.2003

Evaluation of an Enzyme Immunoassay Technique for Detection of Antibodies against *Treponema pallidum*

Rita Castro,[1]* Emília S. Prieto,[1] Irene Santo,[2] Jacinta Azevedo,[2] and Filomena da L. Exposto[1]

Unidade de Doenças Sexualmente Transmitidas, Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical,[1] and Centro de Saúde da Lapa, Ministério da Saúde,[2] Lisbon, Portugal

Received 22 March 2002/Returned for modification 16 August 2002/Accepted 30 September 2002

In the present study, the performance of an enzyme-linked immunosorbent assay (ELISA) technique (Eti-syphilis-G and Eti-syphilis-M; DiaSorin) for detection of *Treponema pallidum* immunoglobulin M (IgM) and IgG antibodies for the laboratory diagnosis of syphilis was evaluated. Four hundred forty-one samples were studied. The sensitivity and specificity of the ELISA were 100 and 93%, respectively, compared with the results of a microhemagglutination assay for *Treponema pallidum* (MHA-TP) and 99.4 and 100%, respectively, compared with the results of the fluorescent treponemal antibody absorption (FTA-Abs) test. The results of the ELISA technique were concordant with those of MHA-TP for 98% of the samples tested, while the rate of concordance with the FTA-Abs test was 99.5%. The sensitivities of the rapid plasma reagin (RPR) test, MHA-TP, and the ELISA in the different phases of syphilis compared with the results of the FTA-Abs test were 92, 88, and 100%, respectively, for patients with primary syphilis; 100% for all tests evaluated for patients with secondary syphilis; 97.2, 99.4, and 100%, respectively, for patients with latent syphilis; and 57.9, 92.6, and 97.9%, respectively, for patients with past treated syphilis. The RPR test was reactive with 12 samples that were negative by all the specific tests. IgM antibodies were most frequently detected by the ELISA for IgM antibodies (32.8%) than by the FTA-Abs for IgM antibodies (28.4%). Detection of these antibodies by the FTA-Abs test and the ELISA for IgM antibodies decreased with the stage of disease (72 and 88%, respectively, for patients with primary syphilis to 17 and 19%, respectively, for patients with early latent syphilis). The high sensitivity and specificity of this ELISA technique during all stages of syphilis, together with the fact that it is a simple, objective, and easily automated method, lead us to believe that it could be used as a screening test for syphilis.

Treponema pallidum subsp. *pallidum,* the etiological agent of syphilis, is a difficult organism to culture (2, 8). Since direct microscopy is possible only when lesions are present, and this is not the case in the majority of patients, detection of antibodies against *T. pallidum* is the most effective method for the diagnosis of syphilis.

The serological tests used most often are the nontreponemal tests (the Venereal Disease Research Laboratory test and the rapid plasma reagin [RPR] test) and the treponemal tests (microhemagglutination assay for *Treponema pallidum* [MHA-TP] and fluorescent treponemal antibody absorption [FTA-Abs] test) (6). The first two methods are generally used to screen large numbers of samples and are sensitive, relatively easy to perform, and inexpensive. However, they are nonspecific and react with lipoid antigens resultant from cellular destruction or from other treponemal species, and as a consequence, false-positive reactions may occur. The rates of these reactions may reach almost 50% (5) for low-risk populations, and therefore, the results must be confirmed by treponemal tests.

Enzyme immunoassays have shown some advantages in relation to the tests used for the laboratory diagnosis of syphilis (4, 9, 13, 17), since they are easy and quick to perform and objective to read. They also have the potential to be automated.

* Corresponding author. Mailing address: Unidade de Doenças Sexualmente Transmitidas, Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Rua da Junqueira 96, 1349-008, Lisbon, Portugal. Phone: 213652600. Fax: 213632105. E-mail: ritacastro@ihmt.unl.pt.

In the present study, we evaluated an enzyme-linked immunosorbent assay (ELISA) technique for detection of *T. pallidum* immunoglobulin G (IgG) and IgM antibodies in patients suspected of having syphilis in an attempt to establish whether this technique can be used for the routine laboratory diagnosis of syphilis.

MATERIALS AND METHODS

Four hundred forty-one patients attending a sexually transmitted disease clinic in Lisbon, Portugal, were enrolled in the study after informed consent was obtained. They were distributed into five groups, as follows: 25 patients with primary syphilis, 25 patients with secondary syphilis, 179 patients with latent syphilis, 105 individuals with a history of syphilis that had been correctly treated, and 107 individuals with no clinical history of syphilis.

The scientific council of the Instituto de Higiene e Medicina Tropical approved the study since it represents the committee on research with human subjects.

All samples were tested for *T. pallidum* antibodies by the RPR test (MacroVue; Becton Dickinson), MHA-TP (Phasyl 210), and FTA-Abs IgG and IgM (Euroimmune) according to the instructions of the manufacturers.

TABLE 1. Qualitative results obtained by the ELISA and MHA-TP[a]

IgG ELISA result	No. of samples with the following MHA-TP result:		
	Reactive	Nonreactive	Total
Reactive	313	9	322
Nonreactive	0	119	119
Total	313	128	441

[a] Sensitivity (313 of 313 samples), 100%; specificity (119 of 128 samples), 93%; agreement (432 of 441 samples), 98%.

TABLE 2. Qualitative results obtained by the ELISA and FTA-Abs test[a]

IgG ELISA result	No. of samples with the following FTA-ABS test result:		
	Reactive	Nonreactive	Total
Reactive	322	0	322
Nonreactive	2	117	119
Total	324	117	441

[a] Sensitivity (322 of 324 samples), 99.4%; specificity (117 of 117 samples), 100%; agreement (439 of 441 samples), 99.5%.

The enzyme immunoassay (Eti-syphilis-G and Eti-syphilis-M; DiaSorin), including all incubation steps and washings, was also performed according to the instructions of the manufacturer. First, a 1:100 dilution of all serum samples was obtained. Samples of diluted sera (100 μl) were then added to the microwells, which were covered with purified *T. pallidum* antigen. After this procedure, peroxidase-labeled antihuman monoclonal antibodies were added. The chromogenic substrate tetramethylbenzidine was then added to each well. IgG and IgM antihuman monoclonal antibodies were used to differentiate between *T. pallidum* IgG and IgM antibodies. The reaction was stopped after 30 min by adding a stop solution, and then the result was read in a spectrophotometer at 450 nm.

Test validation and interpretation were performed by introducing one negative control and two positive controls two times each. The run was considered valid if the mean absorbance for the negative control was equal to or less than 0.250, if the mean absorbance for one of the positive controls was between 0.300 and 0.500, and if the mean absorbance for the other positive control was above 0.500. According to the instructions of the manufacturer, the cutoff point between a positive and a negative result was set at the absorbance of the low-titer positive control. Screened samples with an absorbance within about 10% of that for the low-titer positive control were retested.

A sample with different results by the various techniques used in this study was retested by a technologist who was unaware of the previous results.

Statistical analysis. The significance of the differences in the results was analyzed by the χ^2 test with Yates' correction.

RESULTS

Among the 441 samples studied, 289 (65.5%) were reactive by the RPR technique, while 152 (34.5%) were nonreactive. By the MHA-TP, FTA-Abs, and ELISA techniques, 313 (71%), 324 (73.5%), and 322 (73%) samples, respectively, were reactive, while 128 (29%), 117 (26.5%), and 119 (27%) samples, respectively, were nonreactive.

All samples were used to evaluate the sensitivity and specificity of the ELISA technique in relation to the results of the other specific tests: MHA-TP (Table 1) and the FTA-Abs test (Table 2). The sensitivities of the ELISA were 100% compared with the results of the first test and 99.4% compared with the results of the second test. The specificities were 93% when the results of the ELISA were compared with those of MHA-TP and 100% when the results of the ELISA were compared with those of the FTA-Abs test. The concordance of results between the ELISA and the microhemagluttination technique was 98%, while that between the ELISA and the FTA-Abs test was 99.5%.

The sensitivities of all serological tests used in this study were also evaluated by using the FTA-Abs test as the "gold standard" and for each group of patients described above (patients with primary, secondary, and latent syphilis and individuals with past treated syphilis). When possible, the specificities were also evaluated (Table 3). For the primary syphilis group, the sensitivities of the RPR test, MHA-TP, and the ELISA technique were 92, 88, and 100%, respectively. For the secondary syphilis group, the sensitivities of all tests evaluated were 100%, while for the latent syphilis group and those patients with past treated syphilis, the sensitivities were 97.2 and 57.9%, respectively, for the RPR test; 99.4 and 92.6%, respectively, for MHA-TP, and 100 and 97.9%, respectively, for the ELISA technique.

When samples from individuals with no clinical signs of *T. pallidum* infection and with a nonreactive FTA-Abs test (supposedly noninfected patients) were evaluated, 12 samples were positive by the RPR test. Samples from this group of patients were nonreactive by both the ELISA and MHA-TP. Consequently, the specificity of the RPR test was 88.8%, while those of the other two methods were found to be 100%. Table 4 describes the data related to the detection of anti-*T. pallidum* IgM antibodies by the ELISA and FTA-Abs techniques in different phases of disease. Twenty-five patients with primary syphilis, 25 patients with secondary syphilis, and 179 patients with latent syphilis were analyzed. Sixty-five of 229 samples (28.4%) were reactive for IgM by the FTA-Abs test, the reactivities of 14 of 229 samples (6.1%) were indeterminate, and 150 of 229 samples (65.5%) were nonreactive. When the ELISA technique was used, 75 of 229 serum samples (32.8%) were shown to be reactive and 150 of 229 serum samples (65.5%) were nonreactive, and the reactivities of 4 of 229 serum samples (1.7%) were considered indeterminate. These differences, however, were not statistically significant. In this study, we have also analyzed samples from patients with past treated syphilis for the presence of anti-*T. pallidum* IgM antibodies. These samples were nonreactive by the ELISA and the FTA-Abs methods.

DISCUSSION

The possibility of using an ELISA technique as an alternative to the treponemal tests has been evaluated in various

TABLE 3. Sensitivities and specificities of RPR test, MHA-TP, and IgG ELISA in comparison to results of FTA-Abs test for IgG

Syphilis stage[a]	RPR test		MHA-TP		IgG ELISA	
	Sensitivity[b]	Specificity[c]	Sensitivity	Specificity	Sensitivity	Specificity
PS	23/25 (92)		22/25 (88)		25/25 (100)	
SS	25/25 (100)		25/25 (100)		25/25 (100)	
LS	174/179 (97.2)		178/179 (99.4)		179/179 (100)	
PTS	55/95 (57.9)		88/95 (92.6)		93/95 (97.9)	
NHS		95/107 (88.8)		107/107 (100)		107/107 (100)

[a] PS, primary syphilis; SS, secondary syphilis; LS, latent syphilis; PTS, past treated syphilis; NHS, no history of syphilis.
[b] The data represent the number of samples positive by the test/number of samples positive by the FTA-Abs test (percent).
[c] The data represent the number of samples negative by the test/number of samples negative by the FTA-Abs test (percent).

TABLE 4. Detection of anti-*T. pallidum* IgM antibodies during early syphilis by the FTA-Abs test for IgM and the ELISA for IgM

Syphilis stage[a]	FTA-Abs test for IgM			ELISA for IgM		
	No. of reactive samples/total no. of samples tested (%)	No. of nonreactive samples/total no. of samples tested (%)	No. of samples with indeterminate results/total no. of samples tested (%)	No. of reactive samples/total no. of samples tested (%)	No. of nonreactive samples/total no. of samples tested (%)	No. of samples with indeterminate results/total no. of samples tested (%)
PS	18/25 (72)	1/25 (4)	6/25 (24)	22/25 (88)	2/25 (8)	1/25 (4)
SS	17/25 (68)	6/25 (24)	2/25 (8)	19/25 (76)	5/25 (20)	1/25 (4)
LS	30/179 (17)	143/179 (80)	6/179 (3.3)	34/179 (19)	143/179 (79.9)	2/179 (1.1)
Total	65/229 (28.4)	150/229 (65.5)	14/229 (6.1)	75/229 (32.8)	150/229 (65.5)	4/229 (1.7)

[a] PS, primary syphilis; SS, secondary syphilis; LS, latent syphilis.

studies. The sensitivities of the tests varied from 48.5 to 100%, according to the type of ELISA, with the phase of the disease, and with the disease prevalence (1, 6, 7, 12, 14, 16).

In this study, the ELISA showed a sensitivity that was similar to those of the tests generally used for the laboratory diagnosis of syphilis. Recently, other investigators using other ELISA techniques obtained results similar to those found in the present study (1, 3, 10, 11, 13, 15).

Although a good specificity of the ELISA was obtained when the results of the ELISA were compared with those of the FTA-Abs test and MHA-TP, the specificity of the ELISA was higher than that of the fluorescence test. The rates of concordance of the results of the ELISA technique with those of the other two tests were also found to be high (99.6 and 98%, respectively).

When the sensitivities of the ELISA, RPR test, and MHA-TP were compared by using the FTA-Abs test as the standard, the ELISA technique had a higher sensitivity, although the difference was not statistically significant, during all phases of disease, although other investigators have found that the ELISA method has a lower sensitivity during the primary stage of syphilis (7, 14). Since detection of anti-*T. pallidum* IgM antibodies is important in the differentiation between recent and late infection, we decided to verify using both the ELISA and the FTA-Abs techniques whether IgM antibodies were present in patients with recent stages of syphilis. A larger number of samples from patients with primary syphilis than from patients with secondary syphilis had IgM antibodies against *T. pallidum*, although the differences were not statistically significant. Samples from patients with latent syphilis presented with less reactivity with these antibodies by both tests. When the results of the ELISA for IgM antibodies were compared with those of the FTA-Abs test for IgM antibodies, the number of samples with indeterminate reactivities was smaller by the ELISA for IgM antibodies than by the FTA-Abs test for IgM antibodies. It seems that the majority of samples with indeterminate reactivities obtained by the latter test were indeed reactive. It should also be mentioned that IgM antibodies were not detected in individuals with treated past syphilis by either the ELISA or the FTA-Abs technique. This fact, together with the higher frequency of these antibodies in patients with primary and secondary syphilis than in patients with latent syphilis, demonstrates the utility of this ELISA as a marker of recent infection.

In conclusion, the results of this study show that the ELISA may be an alternative to the treponemal tests for the detection of *T. pallidum* antibodies, including the presence of IgM, since it has a sensitivity and a specificity similar to those of the most commonly used tests during all stages of syphilis. This was especially true when the ELISA technique was compared with the FTA-Abs technique, which is considered the most sensitive and specific test for the diagnosis of syphilis. The ELISA also had a sensitivity similar to that of the RPR test, having the advantage of presenting no false-positive results. We think that the enzyme immunoassay technique studied here could be used as a screening test, since it is simple, objective, and easily automated.

ACKNOWLEDGMENT

We are grateful to Leonard Amaral for kindly editing this article.

REFERENCES

1. **Borobio, M. V., J. M. Ruiz, and E. J. Perea.** 1990. Evalución de una técnica inmunoenzimática en el diagnóstic de sífilis. Enf. Infec. Microbiol. Clin. **8:**486–489.
2. **Fieldsteel, A. H., D. L. Cox, and R. A. Moeckli.** 1981. Cultivation of virulent *Treponema pallidum* in tissue culture. Infect. Immun. **32:**908–915.
3. **Halling, V. W., M. F. Jones, J. E. Bestrom, A. D. Wold, J. E. Rosenblatt, T. F. Smith, and F. R. Cockerill.** 1999. Clinical comparison of the *Treponema pallidum* CAPTIA Syphilis G enzyme immunoassay with the fluorescent treponemal antibody absorption immunoglobulin G for syphilis testing. J. Clin. Microbiol. **37:**3233–3234.
4. **Hooper, N. E., D. C. Malloy, and S. Passen.** 1994. Evaluation of a *Treponema pallidum* enzyme immunoassay as a screening test for syphilis. Clin. Diagn. Lab. Immunol. **1:**477–481.
5. **Larsen, A. S.** 1989. Syphilis. Clin. Lab. Med. **9:**545–557.
6. **Larsen, S. A., B. M. Steiner, and A. H. Rudolph.** 1995. Laboratory diagnosis and interpretation of tests for syphilis. Clin. Microbiol. Rev. **8:**1–21.
7. **Lefevre, J. C., M. A. Bertrand, and R. Bauriaud.** 1990. Evaluation of the Captia enzyme immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. J. Clin. Microbiol. **28:**1704–1707.
8. **Norris, S. J.** 1982. In vitro cultivation of *Treponema pallidum*: independent confirmation. Infect. Immun. **36:**437–439.
9. **Pope, V., E. F. Hunter, and J. C. Feeley.** 1982. Evaluation of the microenzyme-linked immunosorbent assay with *Treponema pallidum* antigen J. Clin. Microbiol. **15:**630–634.
10. **Pope, V., M. B. Fears, W. E. Morril, A. Castro, and S. E. Kikkert.** 2000. Comparison of the Serodia *Treponema pallidum* particle agglutination, Captia Syphilis-G, and SpiroTek Reagin II tests with standard test techniques for diagnosis of syphilis. J. Clin. Microbiol. **38:**2543–2545.
11. **Reinsner, B. S., L. M. Mann, C. A. Tholcken, R. T. Waite, and G. L. Woods.** 1997. Use of the *Treponema pallidum*-specific Captia Syphilis IgG assay in conjunction with the rapid plasma reagin to test for syphilis. J. Clin. Microbiol. **35:**1141–1143.
12. **Rodriguez, I., E. L. Alvarez, C. Fernandez, and A. Miranda.** 2002. Comparison of a recombinant-antigen enzyme immunoassay with *Treponema pallidum* hemagglutination test for serological confirmation of syphilis. Mem. Inst. Oswaldo Cruz **97:**347–349.
13. **Sambri, V., A. Marangoni, M. A. Simone, A. D'Antuono, M. Negosanti, and R. Cevenini.** 2001. Evaluation of recomWell Treponema, a novel recombinant antigen-based enzyme-linked immunosorbent assay for the diagnosis of syphilis. Clin. Microbiol. Infect. **7:**200–205.
14. **Schmidt, B. L., M. Edjlalipour, and A. Luger.** 2000. Comparative evaluation

of nine different enzyme-linked immunosorbent assays for determination of antibodies against *Treponema pallidum* in patients with primary syphilis. J. Clin. Microbiol. **38:**1279–1282.
15. **Silletti, R. P.** 1995. Comparion of CAPTIA Syphilis G enzyme immunoassay with rapid plasma reagin test for detection of syphilis. J. Clin. Microbiol. **33:**1829–1831.
16. **Yong, H., A. Moyes, A. McMillan, and A. Patterson.** 1992. Enzyme immunoassay for anti-treponemal IgG: screening or confirmatory test? J. Clin. Pathol. **45:**37–41.
17. **Young, H., A. Moyes, A. McMillan, and D. H. H. Robertson.** 1989. Screening for treponemal infection by a new enzyme immunoassay. Genitourin. Med. **65:**72–78.